

AMENDMENTS TO THE SPECIFICATION

On page 18, please replace the paragraph starting with “Briefly” and ending with “purification” with the following amended paragraph:

Briefly, first cells were cultured in CHO-S-SFMII medium (GIBCO-BRL, Paisley, Scotland) supplemented with 1 % ULTROSER[®]G (a serum substitute), 200µg/ml STREPTOMYCIN, 200U/mL penicillin (GIBCO-BRL) and 0.3% antiFOAM a (Heraeus Instruments GmbH) in T culture flasks (180 mm³). After reaching confluency, the cells were harvested by adding 1mM EDTA. The cells (5 x 10⁶/ml) were then injected in the production module of the miniPERM bioreactor and were continuously cultured in this system over a period of two months. The culture medium in the nutrient module was replaced every second day. The medium containing the rGPIb α fragment (His1-Val289) was harvested twice a week, cells were removed by centrifugation and the following protease inhibitors were added to the supernatant: 20µM leupeptin (Sigma, St. Louis, USA), 1mM PMSF (Sigma) and 1mM N-ethylmaleimide (ICN, Ohio, USA). The supernatant was stored at -80 C prior to purification.

On pages 18-19, please replace the paragraph starting with “The recombinant” and ending with “electrophoresis” with the following amended paragraph:

The recombinant protein was purified as described (Schumpp-Vonach, Cytotechnology 1995; 17: 133-41) with some modifications. For purification, immunoaffinity chromatography was used. The anti-GPIb-mAb 12G1 was coupled to CNBr-activated Sepharose[®] 4B (affinity purification beads provided by Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer’s instructions. Before loading on the column, the pooled supernatant harvested from the miniPERM bioreactor was concentrated 5 times using a CH₂PR Concentrator S₁Y₃ (Amicon, USA). The column was washed with TBS, 0.3 mM CHAPS (Boehringer Mannheim) and bound proteins were eluted with 0.1 M glycine-HCl pH 2.8. The pH of the eluted fractions was neutralized immediately by the addition of 1M Tris-HCl pH 9. The fractions containing the rGPIb α fragment were identified in a sandwich ELISA (cr. infra). Peak fractions were pooled, the concentration of the rGPIb α fragment (His1-Val289) was determined using the Bradford kit

(Biorad, Hercules, USA) with bovine serum albumin as a standard and the pooled fractions were stored at -80°C until further use.

Purity of the recombinant fragment in the pooled eluted fraction was evaluated by SDS-polyacrylamide gel electrophoresis.